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ANTIMICROBIAL ACTIVITY OF AQUEOUS AND ETHANOL EXTRACTS OF VIOLET PLANT (*SECURIDACA LONGIPEDUNCULATA* FRES) ON TESTED PATHOGENIC BACTERIA

Junaidu Sanusi [1], Abdulhadi Bawa Jibia [*2], Jamilu Yahaya Runka [3], Sanusi Liadi [1], Aliyu Ahmad Abubakar [1] and Rabi'u Sani Zurmi [4]

Isa Kaita College of Education [1], Department of Biological Sciences [2], Department of Animal health and Production [3], Federal University Dutsin-ma, Katsina State Nigeria
Department of Biological Sciences [4], Federal University Gusau, Zamfara State Nigeria

Keywords:

Violet plant, *Securideca longepedunculata*, Root bark, Ehanol extract, Antibacterial, Isolate, *Eschericia coli*

Correspondence to Author:
Abdulhadi Bawa Jibia

Asst. Lecturer, Federal University Dutsin-ma, Katsina State Nigeria

E-mail: abdulhadi_jibia@yahoo.com

ABSTRACT: This study was undertaken to investigate the antimicrobial activities of aqueous and ethanol extracts of violet plant, *Securideca longepedunculata* leaves and root bark extracts against the pathogenic bacteria isolates of *Salmonella typhi, Escherichia coli and Pseudomonas aeroginosa*. Disc diffusion method was used for antibacterial testing and Tetracycline drug at 250 mg/ml concentration was used as positive reference standards to determine the sensitivity of the strains. The results of the antibacterial activities revealed that the highest growth inhibition of the tested bacteria was recorded as 8.00±1.00mm at 250 mg/ml, followed by 7.67±1.15mm at 250 mg/ml and the least inhibition was 3.00±2.00mm at 100 mg/ml. The minimum inhibitory concentrations for *E. coli* and *S. typhi* were 124 mg/ml and 250 mg/ml respectively. The results showed significant effect ($P < 0.05$) of antibacterial activity; with the constituents of *S. longepedunculata* leaves and root bark extracts been used as potential antimicrobial agents in the management of microbial diseases caused by pathogenic bacteria species which may in turn be an alternative to chemical antibiotics. There is need to carry out sub-acute toxicity and determination of LD_{50} to isolate, identify, characterized and elucidate the structure of the bioactive compounds and mechanism of action of *S. longepedunculata* extracts against many pathogenic bacteria

INTRODUCTION: Diseases caused by microbes are widespread worldwide; the treatment of these infections is mainly based on the use of antibiotics. A number of antibiotics have lost their effectiveness due to development of resistant strains, mostly through the expression of resistance genes [1]. In addition to this problem, antibiotics are sometimes associated with adverse effects including hypersensitivity, immune-suppression and allergic reactions [2].

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Therefore, there is need to develop alternative antimicrobial drugs for the treatment of infectious diseases from various sources such as medicinal plants. Bacteria are of human and veterinary importance such as, *Bacillus cereus* has been implicated in food-borne intoxication [3]. *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cause diseases like mastitis, abortions and upper respiratory complications, [4]. *Streptococcus faecalis* is a pathogenic bacteria commonly found in the intestines of birds [3].

There were common beliefs that certain plants contained potential healing features which characterize them today as antimicrobial principles [5]. Historically, plants have provided good sources

of anti-infective agents in the fight against microbial infections, [6, 7]. But, increased resistance of antibiotics gave rise to demand on finding and developing new antimicrobial agents by focusing on plants derived active principles [8].

Violet plant (*Securidaca longepedunculata* Fres) is a savanna grown, medicinal herb or shrub belonging to the family, Polygalaceae. The plant has twisted bole or slender erect branches that grow up to 30 ft high found in various parts of Western, Northern and Eastern Nigeria, [9], in Malaysia, Guinea, Cuba and several Asian countries [10]. In herbal medicine practice, the aqueous root extract has been used in religious rites due to their psychotropic effects, [11]. Powdered roots are used to treat headache by rubbing them on the forehead. Infusions of the root are used for washing topical ulcers [12].

Roots in small dose (drastic and dangerous in larger doses) are purgative, diuretic, diaphoretic and emetic; used for eye complaints such as conjunctivitis, malaria, venereal diseases, urethral discharges, stomach problems, dysentery, rheumatism, fibrositis, toothache, headache, sleeping sickness, cough, chest complaints, snakebite, and wound dressing, and as an aphrodisiac, taenifuge, vermifuge and expectorant, [13].

This research was attempted to evaluate the antibacterial activity of violet plant *Securidaca longepedunculata* against pathogenic bacteria.

MATERIALS AND METHODS:

Collection, Identification and Processing of Plant Material:

Fresh roots and leaves of *Securidaca longepedunculata* were collected during the month of May, 2013 at 5:30pm-6:05pm from Kudewa, Kurfi Local Government Area, Katsina State Nigeria. The plant was identified by Dr. Auwal Umar of the Herbarium Section, where the Voucher specimen was preserved, in the Botany Unit of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto Nigeria. The plant materials were properly washed under tap water, rinsed with distilled water, dried under shade and pulverized with a pestle and mortar and kept in a transparent sterile polyethene bag at room temperature for use.

Preparation of Extract:

In preparing the extract, [14, 15] methods were used. Two hundred grams (200g), each of dried plant material was extracted by soaking in 1000 ml of ethanol and water (solvent) in 1000 ml of conical flask, and covered with aluminum foil and allowed for 24 hrs. The extracts were filtered and the solvents removed by warming in oven at 40^{o}C for 3 days. The evaporated extract was stored for 48 hours in sterile universal bottles at room temperature.

Percentage yield of the Crude plant extract:

The percentage yield of the extracts was obtained using the following formula in equation

$$\% \text{ yield} = S2 \div S1 \times 100$$

Where S1 is weight of powdered sample (g)
S2 is weight of the extract obtained (g)

Source and Maintenance of Microbial Test Strains:

Stocked isolate of Bacterial strains of *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* was obtained from Post graduate Microbiology Research Laboratory of Usmanu Danfodiyo University Sokoto, Nigeria. The isolate was maintained on nutrient agar slant at 4ºC and sub cultured on nutrient broth for 24hrs prior to testing.

Sterilization of Glassware:

The glass wares were adequately washed with liquid soap and sufficiently rinsed with tap water and distilled water respectively, air dried and sterilized in hot air oven at 160^{o}C for 1hour, while the conical flask were autoclaved.

Preparation of Media:

Preparation of Sarboroud Dextrose Agar (SDA):

The sarbouraud dextrose agar (SDA) was prepared according to manufacturer's instructions, SDA (65g) was dissolved in 1000 ml distilled water and 0.5g streptomycin solution was added to inhibit bacterial growth. The conical flask was plugged with cotton and capped with aluminum foil,

sterilizing using lender autoclave at 121^{o}C for 15 minutes, cooled to 45^{o}C before been poured into sterilized plates and kept at 30^{o}C, as shown by [16].

Preparation of Potato Dextrose Agar (PDA):
Potato dextrose agar (PDA) was prepared according to manufacturer's instructions, 39g PDA was dissolved in 1000 ml of distilled water, the suspension was mixed until completely homogenized and 0.5g of streptomycin was added to inhibit the growth of bacteria. The conical flask containing the media were plugged with cotton wool and capped with aluminum foil, sterilized using lender autoclave at 121^{o}C for 15 minutes, cooled for 45^{o}C and pouring in to sterile plates. The plates were kept at 30^{o}C as described by [16].

Preparation of Nutrient Agar (NA):
Nutrient agar (NA) was prepared according to manufactures instructions; 28g NA was dissolved in 1000 ml of distilled water, the suspension was mixed until completely homogenized. The conical flask containing the media were plugged with cotton wool and capped with aluminum foil. Using [16] procedures, the flask was sterilized using lender autoclave at 121^{o}C for 15 minutes, cooled to 30^{o}C and poured into sterile plates Biotech Laboratories Ltd.

Antibacterial Testing:
Antibacterial testing of the aqueous and ethanolic extracts of leaves and root were determined by disk diffusion method as described by [17].

Bacterial Culture:
Authentic clinical isolated pure cultures of human pathogenic bacteria, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*, were obtained from Microbiology Post graduate Research Laboratory, Usmanu Danfodiyo University Sokoto, Nigeria. All the test strains were confirmed by cultural and biochemical characteristics and maintained in slants bottles for use.

Evaluation of Antibacterial Activity:
This was carried out using disk diffusion method, according to procedure of [16]. This method is a reliable technique for determining the antibacterial activity of plant extracts. Sixteen (16) plates of nutrients agar were prepared in sterile plates. Each of the bacterial isolate was sub-cultured on fresh nutrient plate and incubated at 37^{o}C for 24 hours. Paper disk of 5 mm were also made from filter paper using paper puncher and were put into a 100 ml conical flask. The disk were sterilized, counted and placed into different concentrations; 100mg/ml, 150mg/ml, 200mg/ml and 250mg/ml of leaves and root bark extracts of *S. longepedunculata* already prepared, it was allowed for 24 hrs.

The bacterial culture from the sub cultured nutrient agar plates were used to streak fresh nutrient agar plates completely before the impregnated paper disk from the different concentrations were inoculated, control disk was soaked in a sterile tetracycline and placed at the centre of the plates. The plates were incubated at 37^{o}C for 24 hours. All the inoculated plates were labeled with the name of the bacteria and concentration of the extract of each disk. After 24 hrs the inhibition zone of the plates was read. Plates showing the zone of inhibition were measured with the aid of meter ruler.

Minimum Inhibitory Concentration (MIC):
The bacterial cultures were maintained on nutrient agar plates and recovered for testing by sub-culturing in nutrient broth (Oxoid) and incubated at 37°C for 24 hrs before use, each bacteria culture was diluted 1:100 with fresh sterile nutrient broth, [18]. The bacteria were streaked in a radial pattern on the agar plates [19], which were incubated at 37°C under aerobic conditions and examined after 24 hours. Complete suppression of growth by specific concentration of an extract was declared active [20]. Each extract was tested at concentrations of 0.25, 0.2, 0.15 and 0.1 mg/ml. Tetracycline was used as standard (positive) controls with distilled water free solutions as blank controls. Each test was replicated three times.

Minimum Bactericidal Concentration (MBC):
The minimum bactericidal concentration (MBC) of the plant extracts was determined by the modified method of [21]. The samples were sub-cultured from MIC plates that showed no growth after 24 hours onto a fresh extract-free solid medium and incubated further for 18 to 24 hours. The highest dilution (least concentration) that yielded no single

bacterial colony on a solid medium was taken as MBC.

Statistical Analysis of Data:

The parameters analyzed in all the phases of the study were subjected to statistical analysis. Statistical significance was determined by one-way analysis of variance with SPSS 16.0 Version. $P < 0.05$ considered as significant followed by Duncan's Multiple Range Test to detect significant differences among the means as well as the interactions between the variable.

RESULTS:

Antibacterial activity of *S. longepedunculata:*

The result of antibacterial activity of *S. longepedunculata* is presented in **Tables 1-3**; which showed significant effect ($P < 0.05$) on the tested bacteria at all the concentrations of the aqueous and ethanol leaves extract. Root bark extracts showed no significance effect ($P > 0.05$) in the concentrations of the tested isolates as shown in **Fig.1-3**.

TABLE 1: ANTIBACTERIAL ACTIVITIES OF *S. LONGEPEDUNCULATA* LEAVES AND ROOT BARK EXTRACTS ON *E. COLI*

Extract	Conc. (mg/ml)	Leaf extract M ±SD(mm)	Root bark extract M ±SD(mm)
Aqueous	100	6.00^{a}+ 1.00	4.00^{ab}+ 3.46
	150	5.33^{a}+ 1.53	4.67^{abc} + 1.53
	200	6.00^{a}+ 2.65	4.67^{abc} + 2.52
	250	8.00^{a}+ 1.00	6.33^{abc} + 3.05
Tetracycline	250	6.67^{a}+3.21	8.3^{c}+1.52
Ethanol	100	3.66^{a}+ 1.15	4.33^{ab}+ 2.08
	150	6.00^{abc}+ 1.00	5.00^{ab}+ 3.46
	200	7.33^{abc} + 1.15	6.33^{ab}+ 3.78
	250	7.00^{abc} + 1.00	7.66^{ab}+ 1.52
Tetracycline	250	7.33^{abc} +1.15	7.00^{a} + 2.64

^{a,b,c} Means in a column with different superscripts are significantly different ($P < 0.05$)
Values are means + standard error of three replications

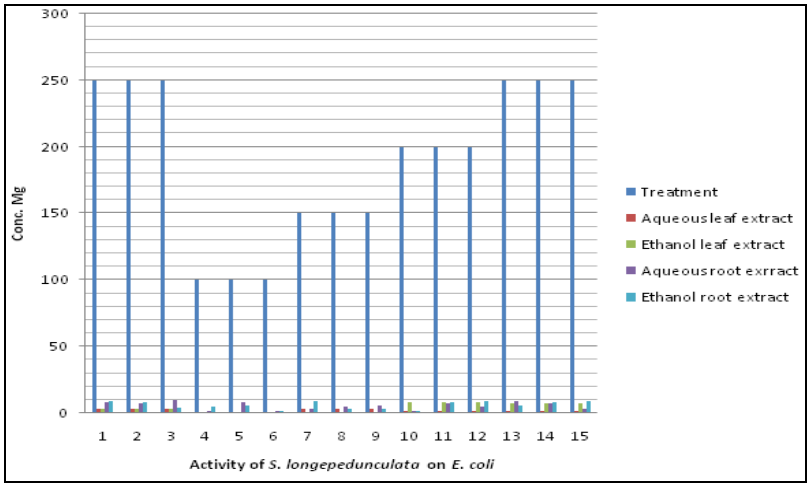

FIG.1: EFFECT OF S. *LONGEPEDUNCULATA* ON *E. COLI*

TABLE 2: ANTIBACTERIAL ACTIVITIES OF *S. LONGEPEDUNCULATA* LEAVES AND ROOT BARK EXTRACTS ON *P. AERUGINOSA*

Extract	Conc. (mg/ml)	Leaf extract M ±SD(mm)	Root bark extract M ±SD(mm)
Aqueous	100	5.00^{ab}+ 1.00	3.33^{a} + 2.309
	150	6.00^{ab}+ 1.00	4.33^{ab} + 1.52
	200	6.67^{ab} + 2.3	6.67^{abc}+3.21
	250	8.00^{ab}+1.00	7.67^{bc}+0.58
Tetracycline	250	7.33^{b}+2.081	8.00^{bc} +1.00
Ethanol	100	4.33^{abc} + .57	8.00^{ab} + 1.00

	150	5.33^{abc} + 1.52	6.67^{ab} +1.53
	200	5.00^{abc} + 4.36	8.00^{ab} +1.00
	250	7.33^{abc} + 1.53	7.67^{ab} + 1.53
Tetracycline	250	7.00^{abc}+2.00	8.33^{b} +1.15

^{a,b,c} Means in a column with different superscripts are significantly different (P < 0.05)
Values are means + standard error of three replications

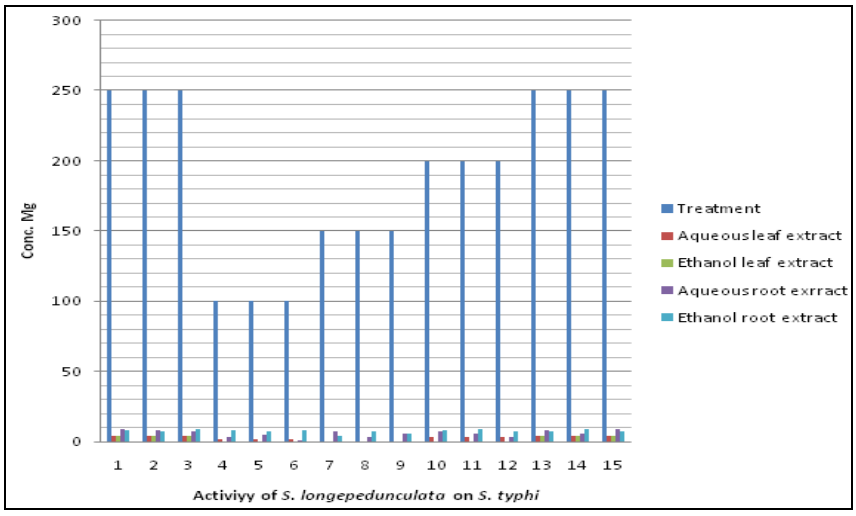

FIG.2: EFFECT OF S. *LONGEPEDUNCULATA* ON *S. TYPHI*

TABLE 3: ANTIBACTERIAL ACTIVITIES OF *S. LONGEPEDUNCULATA* LEAVES AND ROOT BARK EXTRACTS ON *S. TYPHI*

Extract	Conc. (mg/ml)	Leaf extract M ±SD(mm)	Root bark extract M ±SD(mm)
Aqueous	100	4.67^{a}+2.08	3.00^{a} + 2.00
	150	5.67^{a}+2.52	5.33^{abc} + 2.08
	200	6.00^{a}+1.00	5.33^{abc} + 2.08
	250	8.00^{a}+1.00	7.67^{bc}+1.53
Tetracycline	250	8.00^{a}+1.0	8.00^{bc} + 1.00
Ethanol	100	4.00^{ab}+3.61	7.67^{ab} +.57
	150	6.67^{abc}+1.53	5.67^{ab} + 1.5
	200	7.33^{abc}+2.08	8.00^{ab} + 1.00
	250	7.67^{bc}+1.15	7.67^{ab} + 1.15
Tetracycline	250	8.00^{c}+1.00	8.00^{ab} + 1.00

^{a,b,c} Means in a column with different superscripts are significantly different (P < 0.05)
Values are means + standard error of three replications

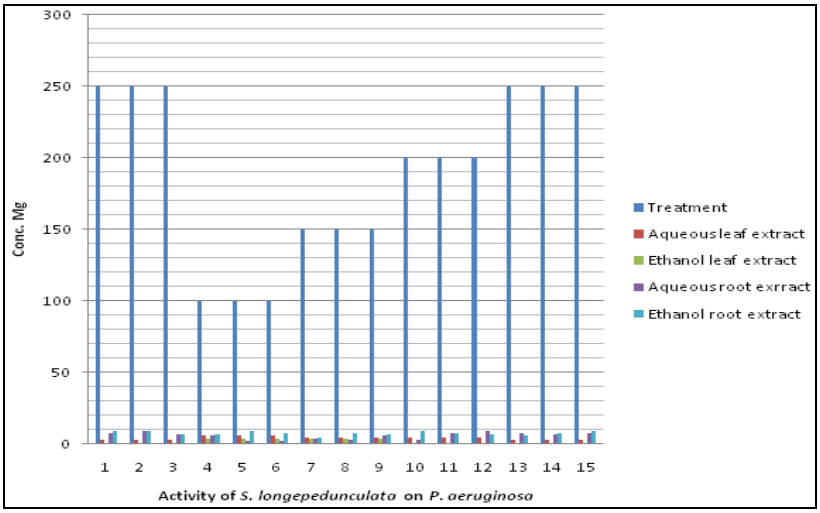

FIG. 3: EFFECT OF *S. LONGEPEDUNCULATA* ON *P. AERUGINOSA*

Minimum Inhibitory Concentration (MIC):
The minimum inhibitory concentration were determined in *E. coli* and *S. typhi* in the different solvent of leaves and root bark extracts while in the *P. aeruginosa* was not found in the aqueous and ethanol leaves and root bark extracts. The highest MIC was observed in *E. coli* of 250 mg/ml on ethanol root bark extracts and the least MIC was observed in *S. typhi* on ethanol leaves extract at 15.65 mg/ml as shown in **Table 4**.

TABLE 4: MINIMUM INHIBITORY CONCENTRATION (MIC) OF *S. LONGIPEDUNCULATA* AGAINST SELECTED PATHOGENIC BACTERIA

Extract	Test organisms	MIC mg/ml
Aqueous leaves extract	*E. coli*	124
	P. aeruginosa	NF
	S. typhi	125
Ethanol leaves extract	*E. coli*	125
	P. aeruginosa	NF
	S. typhi	32
Aqueous root extract	*E. coli*	125
	P. aeruginosa	NF
	S. typhi	16
Ethanol root extract	*E. coli*	125
	P. aeruginosa	NF
	S. typhi	250

NF: Stand for Not Found

DISCUSSION: This research revealed the antimicrobial activity of *Securidaca longepedunculata* in diseases management. There is significant effect ($P < 0.05$) on the tested bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi*) at all the concentrations (100, 150, 200 and 250 mg/ml) of the aqueous and ethanol leaves extracts. Root bark extracts showed no significant effect ($P > 0.05$) in the concentrations, with less activity against the tested bacteria.

The efficacy of the extracts against these bacteria strains might be due to some rich sources of bioactive compounds incorporated in the plant parts. This could also be explained by the fact that *S. longepedunculata* has a medicinal potential with one or more therapeutic usage. These findings are in accordance with the works of [22, 23] who reported that the ethanol extract of *Piliostgma reticulatum* and *Aspila africana* generally displayed highest activities, followed by the hot aqueous and cold aqueous extract against *S. aureus, S. faecalis, P. aeruginosa, K. pneumonia, E. coli, S. dysentriae*, and *S. typhimurium*.

But the result of this study is however in disagreement with the work of [24], where the hot water extracts of root, stem bark and leaf of *Parkia clappertoniana* were more active than their ethanol and cold water extracts against *E. coli* ATCC 11775, *P. aeruginosa* ATCC10145, and *S. aureus* ATCC 12600. The observed antimicrobial sensitivity was highly significance ($P < 0.05$) on the test isolates with reduced diseases incidence as reported in this research.

The significant effect of this violet plant may also arise due acute toxicity of leaf extracts of *S. longepedunculata*. This report indicated greater antimicrobial activities recorded by leaf extract over the root bark extracts, suggesting that more of the bioactive ingredients are lodged in the leaf parts. These report corresponds with the observation of [24] who noticed the significant antibacterial activities recorded by the ethanol and hot water extract of leaf, stem and root extract of *Cyathula prostrata* plant against *E. coli*, *S. aureus*, *P. aureuginosa* and *S. typhi*, are worth-noting especially now that they are multiple-drug resistant species of these bacteria commonly implicated in several cases of human diseases such as gastro-intestinal, urinary tract and wound infections in Nigeria and other African countries [25, 26].

The significant antibacterial activity of this plant extract against these pathogenic bacteria was however in disagreement with the observations of [27], who documented the antibacterial activity of *C. prostrata* and some other traditional Medicinal

Plants (*Leucas aspera*, *Murraya koengigii*, *Oxalis corniculata*, *Alternanthera sessilis*, *Pagostemon benghalensis*, *Hydrocotyl rotendifolia*, *Cyathula prostrata*, *Piper peepuloides*, *Potentilla mooniana*) of North East India on *Escherichia coli*. They noted that the aqueous leaf extracts of *C. prostrata*, *P. benghalensis*, *H. rotendifolia* and *P. mooniana* could not inhibit the growth of *E. coli*. The failure of some extracts to exert antibacterial effect on test organisms in not enough to conclude lack of antimicrobial property because the potency of extracts depends on the solvent and method used to obtain the extract, the age of plant when harvested and the amount of the active constituent, which can vary in quality and quantity from season to season [28].

Antimicrobial activities of *S. longipedunculata* extracts on the growth of bacteria depends on the parts used, solvent of extraction and concentration of the extracts applied, the presence of chemical components of the studied plant parts, the inhibitory zone and concentrations at which values were effective on the tested organism highlight that there is effect of the antibacterial potency of the plant extracts. This could also be attributed to different growth rate of tested organisms, nutritional requirement, temperature and inoculums size [29].

Several reports have shown that antimicrobial activities of extracts from different part of *S. longepedunculata* plant. However the efficacy of the extract to inhibit microbial growth seems to exist according to extraction procedure and assay [29]. This report is in line with the observation of [30] who reported the antimicrobial activities of six ethnomedicinal plants use in the treatment of infectious diseases in which *S. longepedunculata* is included against *E. coli*, *P. aeroginosa*, *S. typhi* and *S. aureus* with significant diameter of inhibition in all the tested organisms ($P < 0.05$).

Antimicrobial activities of ethanol and aqueous extracts of *S. longepedunculata* on root bark and leaves was tested against four gram negative pathogens at four different concentrations, the extracts indicate significant effects ($P < 0.05$) inhibitory activities of aqueous and ethanol extracts. The highest MIC was observed in *E. coli* of 250 mg/ml on ethanol root bark extracts and the least MIC was observed in *S. typhi* on ethanol leaves extract at 15.65 mg/ml. Antimicrobial activities of the ethanol extracts appeared to be more effective then aqueous extracts, since ethanol could extracts a wide variety of active component as compared to aqueous. The presence of chemical compositions in the *S. longepedunculata*, together with the other metabolites which might be present or incorporated, have been reported in this study to show curative activity against diverse pathogens, used traditionally analgesic antimicrobial, anti tumor headache, venereal diseases, constipation and coughs.

The relatively low MIC values recorded by the extracts against the test isolates confirm the high activity of the extract at low concentrations on the toxicity of the ethanolic or aqueous leaf and root bark extract of this plant (*S. longepedunculata*). [31] documented that the Cyathula prostrata extract could be administered at a dose range of 100 mg/kg/BW without any side effects in mice. The presence of bio active compounds of *Adiantum pedatum* at 100 and 200mg/ml and metabolites (cardiac glycosides and steroids) have been documented to inhibit the many bacteria and found to possess antioxidant potentials [32]. High activity of antimicrobial agents at low concentration, in relation to the standard reference drug (tetracycline) concentration is very essential for chemotherapeutic purposes because of the extracts efficacy or toxicity. This ethanolic-aqueous extract is highly active against many disease or pathogenic bacteria.

CONCLUSION: The results of this study confirms the potential uses leaves and root bark of violet plant, *Securidaca longepedunculata* as antimicrobial agent against infectious diseases caused by *E. coli*, *P. aeruginosa* and *S. typhi* for bacteria. The presence of these importance substances suggest that *S. longepedunculata* may possess myriads of therapeutic tendencies and ability to manage numerous health malaises coursed by bacteria. The extracts of plant leaves and root bark used in this research, also demonstrated significant antimicrobial properties on tested pathogenic bacteria; with a high

significant (P<0.05) antimicrobial sensitivity on the test isolates with reduced diseases incidence.

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